Photoperiodism After 50 Years

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The time was midsummer of 1918—the place then was the Arlington Experimental Farm of the U. S. Department of Agriculture near where the Pentagon building now stands. H. A. Allard and W. W. Garner were starting an experiment on Maryland Mammoth tobacco to see if its flowering really depended on the length of the day as their preliminary observations suggested. The results (1) showing the anticipated dependence were soon followed by similar findings on a soybean variety and many other plant species. Through the ensuing years, photoperiodism has been intensively studied and many unexpected ramifications have been found. To mark the 50th anniversary, a symposium on current findings was held by the Agricultural Research Service at Plant Industry Station, Beltsville, Md., on January 26 and 27, 1968.

Three main channels of discovery are now recognized in photoperiodism. The first is the ubiquity of the phenomenon in all plant and animal phyla. The other two are light and time dependencies implied respectively by "photo-" and "periodism." Ubiquity was first sensed by Garner and Allard (1) who wrote ". . . in certain species of red algae, there is a definite periodicity in the appearance of sexual and asexual forms" and ". . . the animal organism is capable of responding to the stimulus of certain day lengths. It has occurred to the writers that possibly the migration of birds furnishes an interesting illustration of this response." It remained, however, for S. Marcovitch (2) in 1924 to prove the point in "The Migration of the Aphididae and the Appearance of the Sexual Forms as Affected by the Relative Length of Daily Light Exposure." He was soon followed by W. Rowan (3) who wrote in 1926, "On Photoperiodism, Reproductive Periodicity, and the Annual Migration of Birds and Certain Fishes." A photoperiodic change in red algae, suspected by Garner and Allard in 1920, was not shown until 1967. The conchocelis phase of *Porphyra tenera* Kjillmn was found by M. J. Dring (4) to be induced by short-day conditions achieved by light interruptions of long-dark periods.

In the 1968 symposium, Dora K. Hayes of the Entomology Research Division, Agricultural Research Service, gave the first precise measurements on the breaking of diapause of insects by light. These results (5) with diapausing larvae of the codling moth (*Carpocapsa pomenella* L.) and the Chinese oak silkworm (*Antheraea pernyi* Guer.) show a maximum response in the blue region of the spectrum, with several subsidiary maxima in the green and yellow and some action in the red near 630 nm. Hayes discussed the way in which light action on the insect brain takes part in the hormonally determined responses leading to eclosion and metamorphosis. Results obtained in 1954 on gamete release from a Coelenterata *(Hydractinia echinata)*, which were discussed by S. B. Hendricks, show a closely similar action spectrum to that of the codling moth and silkworm. Action maxima are in the regions expected for light absorption by a porphyrin.

The symposium dealt mostly with light control of plant development. Attention centered around the action of the blue chromoprotein phytochrome, which was recognized from physiological work in

1952 (6, 7) as determining the light control. Phytochrome (P) is photolabile and can be changed by irradiation from a red (660 nm maximum) to a far-red (730 nm maximum) absorbing form. The far-red form, P_{fr} is physiologically active. An *in vivo* assay, based on the photoreversibility, was devised in 1959 (8) and through its use P was isolated in 1964 (9).

H. Linschitz of Brandeis University gave results of flash excitation of P in which a number of short-lived intermediate forms are observed between $P_r \rightarrow P_{fr}$ and $P_{fr} \rightarrow P_r$. A transient form P_{r_1} with an absorption maximum at 695 nm appears with a first-order rate constant of about 5300 sec^{-1} at 0.6°C (10) when P_r is flashed. This is quickly followed, in half times from milliseconds, by three other intermediate forms before final appearance of P_{fr}. A question exists as to whether these changes take place in series or are parallel in part. Evidence bearing on this point is obtained at low temperatures, between 0°C and —196°C, where the transitions are slowed down. Linschitz concludes that the transitions are parallel in part and that the first intermediate can be held at low temperatures (—196°C) where it is photoreversible to P_r. Anderson, of E. I. DuPont de Nemours Company, stated that in flash excitation, as observed by him, isobestic absorptions are seen between the intermediates, suggesting a series conversion. W. R. Briggs, of Harvard University, described observations on the kinetics of $P_r \rightarrow P_{fr}$ photo-intermediates over a time of many minutes, which is longer than would be expected from Linschitz's results. Conversions of $P_{fr} \rightarrow P_r$ observed by Linschitz took place in milliseconds with two intermediates being involved. There was some speculation, but no actual evidence, that the several intermediates might be involved in physiological display.

Isolated P has been brought to a high state of purity by groups at the Smithsonian Institution (11) and at E. I. DuPont de Nemours and Company (12). Observations of possible multiple forms of isolated P at 25° were described by D. L. Correll, J. L. Edwards, and W. A. Shropshire, Jr. of the Smithsonian Institution. They conclude that the P chromophore can exist in four forms over long periods leading to absorption maxima at 580 and 660 for P_r and 730 nm for P_{fr} (13). These might be involved in the observations made by Briggs (above). Information on the

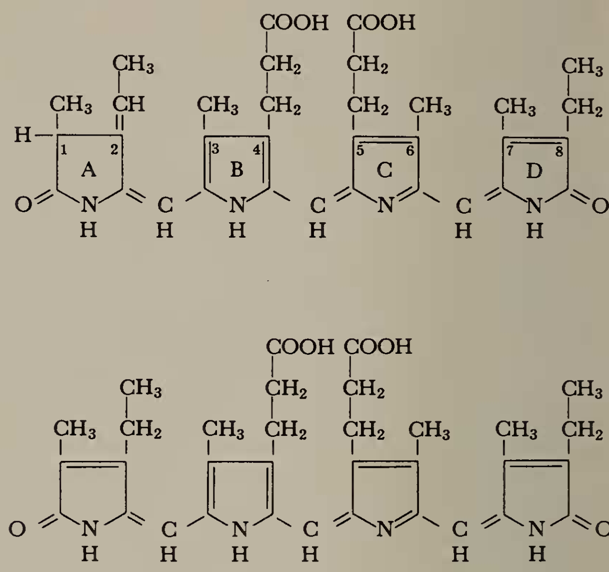

Figure 1. Structure of phycocyanobilin (upper) compared with that of mesobiliverdin (lower).

constitution and behavior of the protein moiety of P was exchanged in discussion by the two groups. One question concerns the presence or absence of sulfur in the protein. There is the eventual hope in this approach of establishing the protein relationship to the chromophore of phytochrome. Linschitz, upon question, stated that the entropy of activation for the first intermediate in flash excitation is of the order of one entropy unit, whereas that for some of the later intermediates is high (> 20 EU), suggesting considerable protein rearrangement.

The possibility that the chromophore of P is related to bile pigments was recognized in 1950 from the action spectra controlling flowering. H. W. Siegelman of Brookhaven National Laboratory described work leading to a full understanding of the structure of the phycocyanin and phycoerythrin bile-pigment type of chromophores (14). These serve as abundant model substances for chemistry related to the still scarce phytochrome. The phycocyanin chromophore, phycocyanobilin, was shown by Siegelman to have the structure shown in Figure 1.

W. L. Butler reported that the optical activity expected from this structure has been observed in his laboratory at the University of California, San Diego. Siegelman speculated that the phytochrome chromophore might have similar arrangements in groups A and D. The phytochrome transformation between the P_r and P_{fr} forms might involve hydrogen migration.

The manners in which many responses of plants are related to phytochrome action were described by Daphne Vince of Reading University, England, M. J. Schneider of Wisconsin University, R. J. Downs of North Carolina State University, H. M. Cathey of the Agricultural Research Service, W. R. Briggs of Harvard University, and W. S. Hillman of the Brookhaven National Laboratory. The responses discussed included flowering of both long- and short-day plants, stem elongation, and tuber formation. These were related to production of agronomic and ornamental plants and to the ecology of seed plants. Flowering and growth responses of many plants to short light breaks of normal dark periods show the controlling function of phytochrome. A more secure basis for P involvement is reversibility found for a potentiated response by far-red light. Survival in the wild and best use of plants in culture are closely dependent on adaptation to the photoperiodic conditions imposed by the season and the latitude.

Agronomic use of photoperiodism depends chiefly on breeding of varieties for limited latitudes. Wheat, maize, sorghum, and soybean varieties have been selected with respect to latitude against photoperiodism as a leading background factor. In the ornamentals industry, chrysanthemum production depends fully upon control of day length, both in reducing long days by darkening of plants to promote flowering and by light breaks during long nights to maintain vegetative conditions. Light routines are carefully assessed for use with other ornamentals (bedding plants, carnations, and azaleas) in combination with growth—modifying chemicals and aspects of management. Competition and persistence of species in open fields and in forests under natural conditions deeply involve photoperiodic responses to an extent that is still poorly assessed.

The photoperiodic control of flowering and stem elongation of long-day plants is much less understood than are those phenomena for short-day ones. Control through phytochrome depends markedly upon the previous main light period, the duration of the night—interrupting irradiation, and the interval chosen for the exposure. Preferred experimental long-day plants have been darnel *(Lolium temulentum)*, henbane *(Hyoscyamus niger)*, and duckweed *(Lemna perpusilla)*. The first of these has the merit of flowering induction by a single long night for one selection. *L. perpusilla* 6746 is very small and can be handled in sterile culture. Hillman found that *L. perpusilla* flowering is favored by the presence of a high level of P_{fr} during one part of the daily cycle and a low level during another part. Blue light (15), which maintains an intermediate level of P_{fr}, can act either like red light (producing predominate P_{fr}) or far-red light (low P_{fr}) depending on the situation. While the observed flowering responses of *L. perpusilla* can be fully accounted for by phytochrome action, responses of several long-day plants (Vince) appear to require some further light action in the blue part of the spectrum as contrasted with far-red.

Control of dormancy was discussed by P. F. Wareing of the University College of Wales. Some woody plants grow continuously on long days. As days shorten, the vegetative buds form a number of scales and become dormant. This dormancy is usually broken only by a period of a month or more at temperatures below 40°F. In a few cases, returning to long-day conditions causes resumption of growth. The response is controlled through the leaves. It is a leading factor in the growth and overwintering of trees in temperate climates and in many of the plant growth features accompanying autumn.

Wareing and his associates extracted an active compound from leaves of the European sycamore *(Acer pseudo-platanus)* that is effective in inducing bud dormancy. It has been given the trivial name abscisic acid (ABA). ABA has been isolated and synthesized by teams of workers in both England (16) and the United States (17). It is a sesquiterpene acid with the formula shown in Figure 2.

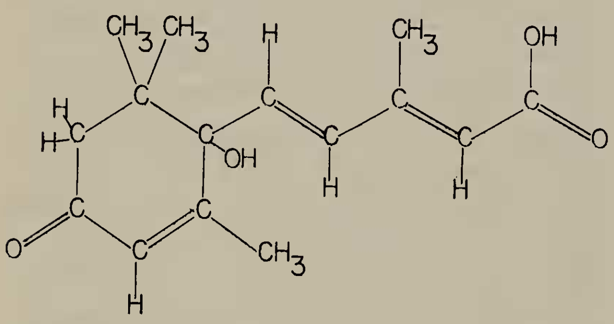

Figure 2. Structure of abscisic acid (ABA).

The cis, trans isomer is the active form. ABA accumulates in the buds of woody plants as the days become short in the autumn. It induces the dormancy or cessation of growth, causes abscission of leaves, inhibits flowering of long-day plants and promotes the flowering of some short-day plants. The concentration of ABA in a bud decreases throughout the winter such that growth can be resumed under the favorable long days of spring. ABA is one of the short, but growing, list of isolated, identified, and synthesized plant hormones. It interplays with gibberellic acid, kinetins, and auxins in its several functions.

Seeds also show pronounced dormancies which resemble diapause in insects as well as bud dormancies in principle. Such dormancies were discussed by A. L. Mancinelli of Columbia University. Many seeds after a period in storage or overwintering in the soil require light to germinate. This light action is a response to change of phytochrome from the P_r to P_{fr} form. Dormant seeds with phytochrome in the P_r form are known to have remained viable in soil for more than 16 centuries (18). They germinated quickly upon exposure to light. Many seeds are also suppressed in germination by prolonged exposures to light. This involves phytochrome action in part as well as some further light action which has come to be known as the high-energy reaction or the HER.

The nature of the HER is under debate at this time. H. Mohr of the University of Freiburg has measured its effect particularly as a control of stem lengthening and other aspects of growth of etiolated seedlings upon exposure to light. A maximum of light action is usually found near 720 nm in the far-red part of the spectrum. Action is also present in the blue part of the spectrum. Because of the position of the far-red action maximum and the effects of simultaneous irradiation of mustard seedlings *(Sinapis alba)* with two wavelengths of radiation in the 600 to 800 nm regions, Mohr (19) considers the HER to be an aspect of phytochrome action.

Control of flowering of the long-day plants, spinach, annual sugarbeet, and henbane were shown by M. J. Schneider to depend upon the HER as well as phytochrome action. H. A. Borthwick and S. B. Hendricks presented results on control of germination of *Ameranthus arenicola* seeds. They interpret their results and previous findings on control of flowering, stem elongation, and anthocyamin formation as an HER display. They differ from Mohr in considering the HER to depend upon a previously unobserved pigment rather than phytochrome. Measurements of absorption spectra of turnip seedling tissue known to display an HER as control of anthocyamin production gave evidence of a weak absorption near 720 nm (Norris).

A role of phytochrome in control of enzyme synthesis was discussed by H. Mohr. He has found that the level of phenylalanine-deaminase activity (20) in mustard seedlings is enhanced by exposure to far-red radiation. He interprets this result as arising from gene depression as a consequence of phytochrome action.

The nature of the first or early processes in phytochrome action has been under discussion during recent years. Mohr (20) holds that gene derepression is such a process. Others have pointed out (21) that an approach to the early action can be found only in very quick responses to the change in form of phytochrome. The natures of several rapid responses suggest that modification of membrane behavior is involved.

T. Tanada reported on the rapid photoreversibility involved in adherence of root tips to glass. This response to phytochrome is obtained only in the presence of indolacetic acid (10^{-9}m), adenosine triphosphate (10^{-6}m), ascorbic acid (10^{-6}m), and several inorganic ions that are known to influence membrane permeability.

Time measurement in photoperiodism—the "Periodism"—is thought to involve the "biological clock" of the organism. The display of the circadian rhythm of several ecological types of *Chenopodium rubrum* under many conditions of long dark periods was reported by B. G. Cumming of the University of Western Ontario. Induction of flowering depends cyclically on the length of the dark period irrespective of whether a light break is used at various hours or plants are returned to continuous light. The rhythm is shown by plants maintained on glucose or sucrose—that is, plants not strictly dependent on photosynthesis—although it damps out after about 72 hours in darkness. In work of this type, it is essential to deal with large plant populations. The *C. rubrum* ecological types used by Cumming are ideal in this respect. Varieties can be selected that flower after a single long night. Flowering can be observed 7 days after planting the seed.

Development of knowledge about photoperiodism has depended chiefly on use of light with appropriate biological material. The great advantage of photostimulation in studies of causation is that the initial act is fixed as a single photoexcitation irrespective of complexities of later expression in flowering, stem elongation, or seed germination. Measurements of action spectra, expressing the energies required at various wavelengths for a given response, have formed the basic procedure in studies of photoperiodism. These led to the discovery of phytochrome and to its photoreversibility. Optical devices are used in physical assays for phytochrome. Results of flash excitation are followed by measurements of light absorption in periods as rapid as milliseconds to detect short-lived intermediates.

K. H. Norris of the Agricultural Research Service described various spectroscopic and light-measuring devices for use in photoperiodic work. Among these was a simple spectrometer made with one or two wedge interference filters. He also described the measurement and analysis of absorption spectra for detection of extremely minor constituents. These methods were applied to detection of possible absorbing compounds in the spectral region involved in the HER.

Advances in knowledge of photoperiodism during the last 50 years have increased practical use and have brought the more basic questions to a point of reasonable study. Among these questions are the exact character of the first biological change induced by phytochrome. Another question, now amenable to study, concerns the interplay of hormonal activities in both plants and animals in their dependence on environmental factors, chief among which is the length of the day. There is hope of better understanding the determinative steps in biological rhythms. More remote, but still involved in the photoperiodic responses, is development of an understanding of control of differentiation, as expressed in flowering, and of structure elongation and expansion.

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